strain of *P luminescens* (W14) and then tested the effect of the mutant bacterial broths in our oral bioassay. Deletion of either *tca* or *tcd* individually (as tca^- or tcd^- mutant strains) greatly reduced the oral toxicity of the broth to *M sexta*, whereas deletion of both *tca* and *tcd* together (in the tca^-/tcd^- double mutant) eliminated oral toxicity altogether. These results suggest that both Tca and Tcd are involved in oral toxicity to Lepidoptera. However, we have been unable to purify sufficient quantities of Tcd to perform an LD_{50} determination.

In order to examine the effects of Tca on the lepidopteran gut and compare it to that previously documented for both the *B thuringiensis* δ-endotoxins and vegetative insecticidal proteins (Vips), and for cholesterol oxidase,[11–15] we sectioned *M sexta* neonates at intervals after oral ingestion of toxin. After several hours, toxin-treated midguts showed an accelerated rate of epithelial blebbing (Fig 2). This blebbing of the midgut epithelium into the lumen continues until the basement membrane is exposed and the epithelium is essentially destroyed. Both the columnar cells and the goblet cells appear to be attacked. Interestingly, a similar histopathology can be observed following injection of Tca directly into the insect haemocoel, which is presumably the normal route of delivery of the toxin by the bacterium.[16]

In conclusion, we have purified four toxin complexes from the culture broth of *P luminescens* and cloned the four toxin complex-encoding loci. Genetic knockout of either *tca* or *tcd* reduces oral toxicity to *M sexta* and knockout of both loci eliminates activity. Purified Tca shows effects specifically on the insect midgut, despite its putative normal delivery directly into the insect haemocoel. These *Photorhabdus* toxins (Phts) may form useful alternatives to other orally active bacterial protein toxins such as those from *B thuringiensis* (Bt).

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Synthesis of a biotin-like phosphonate model compound for (+)-hydantocidin

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Abstract: Approaches to the synthesis of a biotin-like phosphonate are described. It was hoped that this would be a simpler model compound for the

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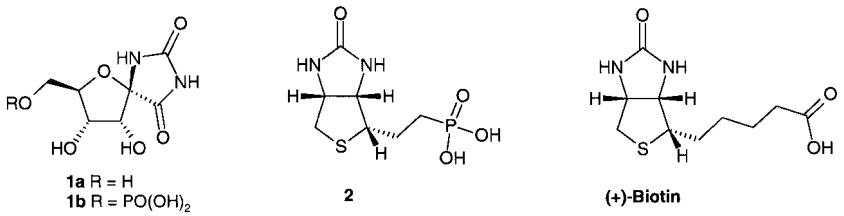

Figure 1. Structures of (+)-hydantocidin (**1a**) and possible simpler model compounds.

naturally occurring spironucleoside (+)-hydantocidin, but it showed no activity as a herbicide nor as an inhibitor of AdSS.

Keywords: (+)-hydantocidin; model compound; phosphonate; biotin-like

1 INTRODUCTION

(+)-Hydantocydin (**1a**; Fig 1) is a naturally occurring spironucleoside with potent herbicidal activity and is produced by certain strains of the actinomycete *Streptomyces hygroscopicus* (Tü 2474).[1] It is actually a pro-herbicide, in that the corresponding 5′-phosphate (**1b**) inhibits adenylosuccinate synthetase (AdSS) in de-novo purine biosynthesis.[1,2]

The presently known synthetic methods for the parent compound **1a** are not economically feasible and its close analogues lack biological activity.[1] Since X-ray data of the enzyme-inhibitor complex **1b** became available, the search for AdSS inhibitors has focused upon the CAMM-supported design of simpler model compounds: as an example, the biotin-like phosphonate **2** fulfils the steric requirements for an AdSS inhibitor.

Racemic **2** was synthesised in seven steps from the phosphonium salt **3a** (Fig 2), a close analogue of a recently identified biotin process key intermediate,[3] by using Wittig methodology for the introduction of the side chain (**5**) followed by appropriate functional group transformations and deprotection. In view of the well-known vigorous *N*-debenzylation reactions in biotin chemistry, a parallel synthesis using *para*-methoxybenzyl as a more-easily cleaved *N*-protecting group was carried out using **3b** as starting material. The choice of protecting group proved to be critical. The key intermediates **3** are readily produced in two steps from thiolactones **4**.[3–7]

2 SYNTHESES

2.1 Introduction of side chain (Fig 3)

Wittig reaction of phosphoranes derived from *rac*-**3** with TBDMS-protected 2-hydroxy acetaldehyde[8,9] competed with reductive formation of thiophanes **6** and **7**. The choice of base was crucial. Best results on a preparative scale (20 mmol) were achieved in the benzyl series with solid $KN(TMS)_2$ in the presence of a catalytic amount of 18-crown-6,[10] thus affording the exocyclic allylic silyl ether **5a** in 66% yield (*E*-configuration predominant), accompanied by the thiophane **6a**.[11] On the other hand, an analogous Wittig reaction with **3b** under similar conditions proceeded disappointingly, **6b** and **7b** becoming the major components due to migration of the neighbouring *p*-MeO-phenyl moiety into the 2α-position. Thus

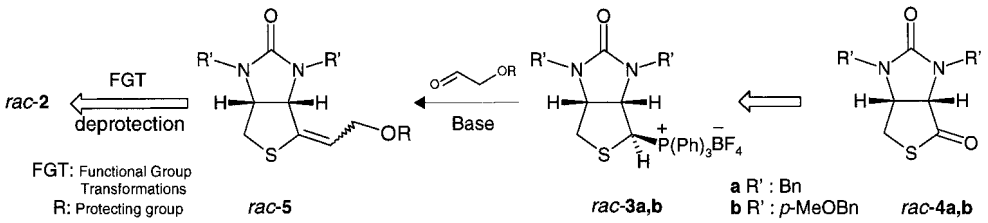

Figure 2. Overall synthetic approach to model compound **2**.

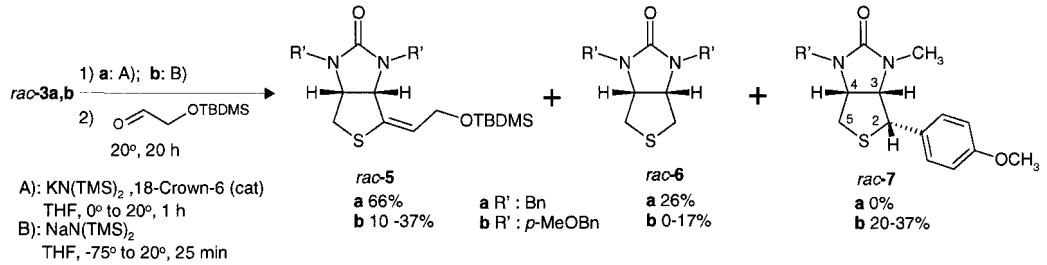

Figure 3. Introduction of side-chain.

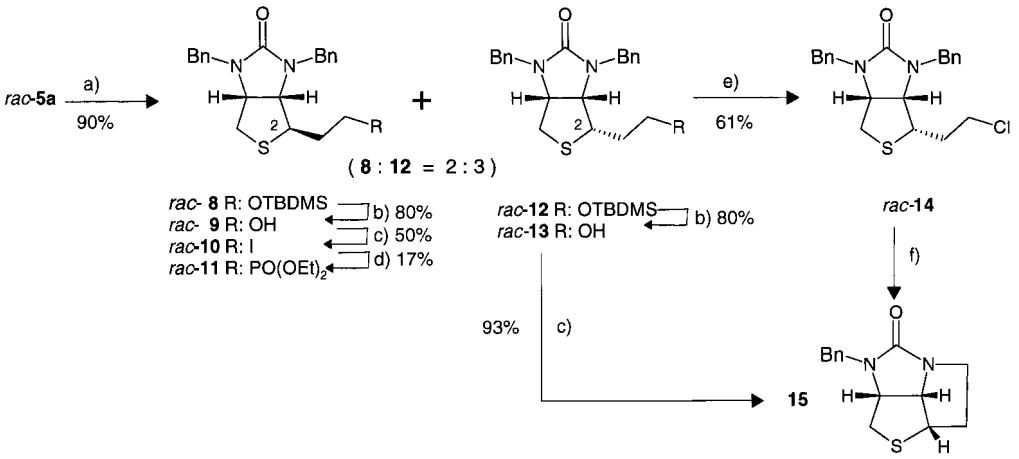

Figure 4. Side-chain transformations, I. (a): H_2, 10% Pd-C, MeOH, 10 bar, 50°, 18h; (b): TBAF, THF, 0°, 3.5h (c): $CH_3P(OPh)_3^+ I^-$,2,6-Lutidin, DMF, 20°, 1.5h; (d): $HPO(OEt)_2$, $KN(TMS)_2$, THF, −70° to 20°, 20h; (e): $P(Ph)_3$, CCl_4, 20°, 20h; (f): $P(OEt)_3$, 130°, 16h, tlc -experiment.

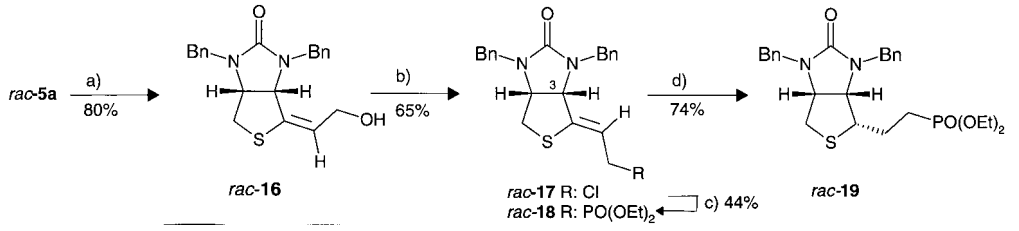

Figure 5. Side-chain transformations, II. (a): Tetrabutylammoniumfluoride, THF, 0°, 3h; (b): $P(Ph)_3$, CCl_4, 75°, 20h; (c): $P(OEt)_2(OTMS)$, 100°, 6h; (d): H_2, 10% Pd-C, MeOH, 10 bar, 50°, 18h.

the *p*-Meo-benzyl series was abandoned. Treatment of **3a** with base alone furnished **6a** as the sole product.

2.2 Side-chain transformations 1 (Fig 4)

Catalytic hydrogenation[4,12] of olefin **5a** led to the mixture of epimers **8** and **12** in good yield, though with poor diastereoselectivity in favour of **12**. Chromatographic separation (elution with diethyl ether+ hexanes, 1 + 1, by volume) and subsequent cleavage of the silyl ethers to alcohols **9** and **13**, posed no problems. The configuration at C-2 of either epimer was assigned on the basis of [^{1}H]NOE experiments.

Further treatment of **13** with methyltriphenoxyphosphonium iodide[13] to obtain the corresponding iodide (as precursor for phosphonylation) afforded exclusively the hitherto-unknown tricycle **15** (a 5-thia-1, 2-diaza-cyclopenta[*cd*]pentalene system) as a viscous oil. With chloride **14** under Arbuzov conditions no phosphonate was observed but it again gave **15**. In contrast, the epimeric 2β-alcohol **9** reacted readily to give the iodide **10**, from which the 2β-phosphonate **11** was isolated and characterised.

The results in the 2α-series may be explained in terms of neighbouring group participation. The close proximity of a benzyl group to the phosphonium intermediates (same type in both reactions) favours attack of halide ion at the benzylic carbon, resulting in ring closure and elimination of benzyl halides.

These facts suggest a reversal of the reaction order in the side-chain transformations with phosphonylation at the allylic stage.

2.2 Side-chain transformations 2 (Fig 5)

Deprotection of silyl ether **5a** under standard conditions afforded the allylic alcohol **16** in good yield. Transformation to the chloride **17** proceeded cleanly but with reversal of configuration of the double bond (*Z*; NOE between olefinic *H* and *H*-3). Subsequent Arbuzov reaction gave the respective *Z*-configured allylic phosphonate **18**. In contrast to *E*-configured **5a**, catalytic hydrogenation[4,12] of (*Z*)-phosphonate **18** proceeded with high diastereomeric excess, resulting in our proposed fully protected model 2α-phosphonoethylthiophane **19**. Traces of 2β-**19**, detected in [^{1}H]NMR, were identical with **11**.

2.3 Deprotection (Fig 6)

Deprotection of **19** proved to be a difficult task. After numerous failures, debenzylation was achieved by simple treatment of **19** with concentrated sulfuric acid overnight at ambient temperature. Column chromatography on silica gel allowed purification and separation from regioselectively monodeprotected **20** to give the crystalline diethyl phosphonate **21** (mp 128–133 °C) in moderate yield. Finally, the pure racemic phosphonic acid **2** was obtained as a solid (mp 240–275 °C dec) after treatment with excess TMSBr and an aqueous work-up.

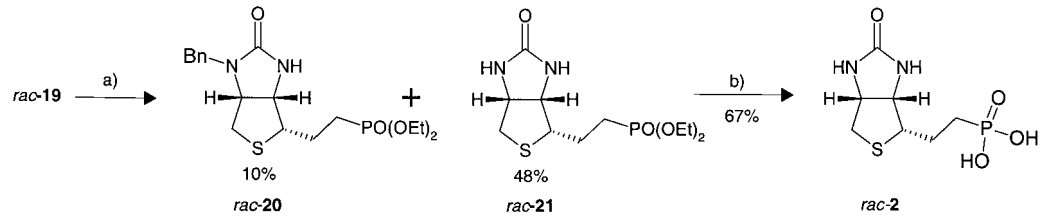

Figure 6. Deprotection. (a): conc.(96%) H_2SO_4, 20°, 16 h; (b):(1) TMSBr (15 equiv.) DCM, 20°, 2.5 days, (2) H_2O.

3 BIOLOGICAL ACTIVITY

Tested at 250 μM **rac-2** and **rac-21** were inactive as inhibitors of adenosylsuccinate synthetase (AdSS).[1]

Both compounds were also inactive when tested as herbicides against a selection of mono- and dicotyledonous weed species at 2 kg ha^{-1} in the Novartis 1a standard screen.

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Plant cell cultures as a model for the biochemistry of crop selectivity

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Abstract: Plant cell cultures have been used to study the metabolic degradation of 4-amino-5-methyl-2-(*tert*-butylaminocarbonyl)-1,2,4-triazolin-3-one. The biochemical basis of selectivity was shown to reside in effective metabolic conjugation. The herbicide was eliminated from cell cultures of beet (which is tolerant to it) by conjugation (*N*-glycolisation), but this occured to only a limited extent with cell cultures of non-target plants such as soybean.

Keywords: crop selectivity; cell cultures; herbicide metabolism

Plant cell suspension cultures are well suited for the study of the metabolic degradation of xenobiotics.[1–3] Here, studies on 4-amino-5-methyl-2-(*tert*-butylaminocarbonyl)-1,2,4-triazolin-3-one (**1**; see Fig 1), which is selective in sugar beet and shows broad-spectrum weed control against monocotyledenous and dicotyledenous species, using plant cell cultures, are presented. The aim of the studies was (i) to establish the pathway of metabolization in target (red beet) and non-target (soybean) plant cell suspension cultures, (ii) to investigate the biochemical basis for tolerance in the target cell culture and for selectivity between target and non-target cell cultures, and (iii) to compare the in-vitro results with the field situation.

Either dark-grown heterotrophic suspension cultures of soybean (*Glycine max* Merr cv *Merill* v *Mandarin*) or light-grown heterotrophic suspension cultures of red beet (*Beta vulgaris conditiva* Alef) were grown in Gamborg B5 medium containing sucrose, (20 g litre^{-1}), and 2,4-D, (0.1 mg litre^{-1}). The ^{14}C-radiolabelled compound was applied in acetonitrile solution. Metabolites were isolated from either the nutrient medium or the cell extract (acetonitrile + water, 8 + 2 by volume), both after partitioning against

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